Examiner Tran is also thanked for indicating the allowability of claims 29 and 30. It is believed the entire application is in condition for allowance for the reasons set forth below.

The 35 U.S.C. § 103(a) rejection of claims 23-28 and 31 over U.S. Patent No. 5,591,453 to Ducheyne et al. in view of International Patent Publication WO 92/20623 to Einarsrud et al. is respectfully traversed. A feature of the claimed method of administering a biologically active agent into a human or animal body is the complete dissolution of a silica-xerogel carrier within a desired time period upon contact with body fluid. See Fig. 1, which illustrate the controlled release of a small biologically active molecule from a silica xerogel carrier. Release of the molecule and dissolution of the carrier are almost linear, which would not be the case if diffusion was the primary release mechanism.

The cited combination of references fails to raise a *prima facie* case of obviousness against the claimed method because the cited references fail to disclose or suggest the complete dissolution of the silica-xerogel feature of the claimed method. None of the Ducheyne et al. examples indicate that its silica glass

carriers completely dissolve when in contact with simulated body fluid.

One of ordinary skill in the art would understand the Ducheyne et al. carrier does not have to dissolve completely to achieve release of its biologically active agent. Instead, Ducheyne et al. teaches that a biologically active agent may be controllably released primarily by diffusion through the pores of its silica-based glass. See, for example, Col. 6, lines 15-17 ("In the case of pure silica glass, the release of the biological molecules from the carrier is effected primarily by diffusion through the pore structure"), Col. 9, lines 16-18 ("Because of the controllable microporosity, a subsequent controlled release of molecule is achieved."), and Col. 14, lines 38-39 ("Larger pore sizes facilitate the release of larger molecules through diffusion.") (Emphasis added). Since the primary release mechanism is taught to be diffusion, biodegradation of the Ducheyne et al. carrier is not required for release.

The Patent Office argues it would be obvious to modify the porosity of the gel to obtain a desired release rate. However, porosity is not a key factor governing the dissolution of a silica monolith, as demonstrated by Viitala et al., "Adjustably

Bioresorbable Sol-Gel Derived SiO_2 Matrices for Release of Large Biologically Active Molecules," 36 J. Sol-Gel Sci. Tech. 147-156 (2005)(copy attached). Fig. 2 and Tables 2 and 3 show that a first SiO_2 monolith (Bm12) can dissolve faster than a second SiO_2 monolith (Am01), even though the first monolith (Bm12) has a specific surface area or a pore volume that is less than one hundredth of the second monolith.[1]

One of ordinary skill in the art, having read Ducheyne et al., would be concerned with controlling the rate of release of a biologically active agent by diffusion of the agent through the silica glass, and would not be given any reason to consider controlling the biologically active agent's release rate by controlling the biodegradability of Ducheyne et al.'s silica glass carrier.

The deficiencies of Ducheyne et al. are not remedied by the additional disclosure of Einarsrud et al., which also fails to disclose or suggest the complete dissolution feature of the claimed

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[1]Like porosity, the chemical composition of a silica-xerogel does not primarily determine its biodegradability. Instead, the dissolution rate is mainly governed by the structure of the continuous silica network, which itself is dependent upon the parameters of the method used to produce the silica xerogel (Specification, page 8, lines 1-10).

method of administering a biologically active agent into a human or animal body. Instead, Einarsrud et al. discloses gels suitable for insulation, and its main emphasis is on obtaining gels with high porosity. As discussed above, porosity is not a key factor in determining the dissolution rate of a silica xerogel monolith.

Reconsideration and withdrawal of the obviousness rejection of claims 23-28 and 31 over Ducheyne et al. in view of Einarsrud et al. are earnestly requested.

It is believed this application is in condition for allowance. Reconsideration and withdrawal of the rejection of claims 23-28 and 31, and issuance of a Notice of Allowance directed to claims 23-31, are earnestly requested. The Examiner is urged to telephone the undersigned should she believe any further action is required for allowance.

A Petition and fee for a one month Extension of Time are attached. It is not believed any additional fee is required for entry and consideration of this Request. Nevertheless, the

PATENT

U.S. Appln. S.N. 10/828,351
REQUEST FOR RECONSIDERATION

Commissioner is authorized to charge our Deposit Account No. 50-1258 in the amount of any such required fee.

Respectfully submitted,

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Enclosures:
Petition for Extension of Time
Viitala et al., "Adjustably Bioresorbable Sol-Gel Derived SiO_2 Matrices for Release of Large Biologically Active Molecules," 36 J. Sol-Gel Sci. Tech. 147-156 (2005)

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Adjustably Bioresorbable Sol-Gel Derived SiO_2 Matrices for Release of Large Biologically Active Molecules

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Abstract. Amorphous, sol-gel derived SiO_2 are known to biocompatible and bioresorbable materials. Bioresorbable materials have potential applications as implants or injectable matrices in the controlled delivery of biologically active agents in the living tissue. Bioresorbable matrices provide desirable properties, e.g., extra removal operations that have to be done with biostable matrices are avoided and the release of large therapeutic molecules can be controlled by matrix degradation rather than by diffusion. New important groups of drugs, such as biotechnically-produced peptides and proteins, are potential to be encapsulated in bioresorbable SiO_2, because they are typically

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larger in size and their direct oral administration without protecting matrix is difficult due to digestion. The methods to achieve a wide range of SiO_2 bioresorption rates (from days to months) are introduced in this study. This is done by a "conventional" alkoxy-based sol-gel method at protein-friendly conditions by adjusting the precursor ratios, aging of the sol and by using different preparation methods (casting, spray-drying and freeze-drying). The prepared morphologies include implantable monolithic sticks and injectable microspheres. The importance of chemical structure is shown in comparison with the specific surface area and pore volume.

Keywords: sol-gel technique, xerogel, bioresorption, porosity, silica

1. Introduction

Tissue-friendly sol-gel derived SiO_2 xerogels are potential biomaterials to be used as matrix materials for controlled release of different kind of biologically active agents, such as conventional small-sized drug molecules [1–4] or for a growing number of new biotechnical drug molecules, such as peptides and proteins [5–8]. Biotechnically-produced drugs need new administration technologies that have a key role in future. Proteins could be used as vaccines, sustained release of growth factor proteins could regenerate damaged tissue, or they could be used as such in targeted areas to replace the function of a twisted protein. The commonly used oral administration is impossible due to degradation in the normal digestion system and other administration technologies are needed. In some cases it would be beneficial to administrate proteins as such locally to desired tissue, e.g., by injection or implantation. To avoid repeated injections and to achieve sustained and controlled release, biocompatible and bioresorbable biomaterials provide a potential solution. Biomaterial carriers could also work as a protecting matrix for proteins to avoid too early degradation in oral administration.

The term bioresorbable is often used to describe materials degradation in or in contact with the living organism, mostly for implanted biomaterials in living tissue describing a removal of liquid phase degradation products by cellular activity. The administration with these matrices have three main challenges to overcome: they should be biocompatible, the release rates of molecules should be controlled on a large scale and the molecules should retain their biological activity during and after the encapsulation. Local administration for targeted therapy requires also preparation of different morphologies, e.g., small stick-formed implants or injectable microspheres with microinvasive properties (minimal damage to tissue). The desired release rates of biologically active agents vary depending on the application and one should be able to adjust the bioresopirion rate from short (days) to longer (months, years) time release.

The sol-gel technology is a flexible method with many advantages. Addition of biologically active agents into silica sol is done in the liquid phase providing possibilities for homogeneous distribution of molecules. Another advantage of the sol-gel method is the low-temperature processing, which is useful, e.g., for proteins. Temperature can be kept low through the whole sol-gel process (commonly within 0–40°C). The silica sol-gel process is strongly dependent on the water-to-silica precursor ratio, pH and temperature, which make it challenging with respect to the peptides and proteins. Although the temperature and pH-window for proteins to retain their activity is quite narrow, also aging of the sol and gel drying can be used to adjust the matrix properties [9].

The potential of SiO_2 xerogel matrices for controlled delivery of small drug molecules have been shown both *in vitro* and *in vivo* [10, 11]. Also proteins encapsulated in SiO_2 matrices have been studied earlier, but in those cases the main focus has been in the use of SiO_2 as a static catalyst carrier matrix for enzymes [12, 13] and in the studies on the activity of the encapsulated molecules [14–19], but not in the release of molecules. The pore diameter of sol-gel derived SiO_2 varies typically from 1 to 10 nm [9] and the out-diffusion of large biologically active molecules is size-controlled. In a such case adjustable bioresorption of SiO_2 xerogel has an important role.

Bioresorption can be homogenous (bulk) or heterogeneous (surface). The surface degradation or surface erosion occurs at the surface of the material. The bulk degradation is the most common matrix degradation mechanism [20] where the materials degrade homogeneously throughout the matrix. The degradation of matrices is often a combination of these mechanisms. The bioresorption of the silica matrix depends both on the chemical and pore structure of the matrix. Although

there are lot of possibilities to adjust the silica pore structure more accurately and to larger pore sizes, e.g. by self-assembling organic additives [21], to match with the protein sizes, the conventional sol-gel method has still advantages. The use of organic additives is challenging, especially *in situ* addition of large and sensitive biologically active molecules. This is due to additional interactions between molecules, biocompatibility of self-assembling amphiphilic molecules and the removal of organic porogen additives (without removal or denaturation of biologically active molecules). Another important factor affecting the matrix degradation is the chemical structure of the SiO_2gel matrix. Low degree of condensation (e.g., by low water-to-silica precursor ratio and pH near 2 [22]) means more free OH-groups on the surface and faster bioresorption. However, the chemical structure affects also the aggregation of colloidal silica particles and aggregate structure and consequently the formed pore structure meaning that there are also challenges in adjusting the bioresorption rate on conventional sol-gel derived silica.

The main aim of this study has been to use protein-friendly pH in the preparation of SiO_2 xerogel matrices and at the same time to retain the possibility to adjust bioresorption (here: degradation *in vitro* by dissolution at the body fluid pH) on a large scale. High temperatures and very acidic or basic pH are avoided because they may denature proteins. Bovine serum albumin (BSA) is used as a model protein. By changing the sol composition, precursor concentrations, pH, temperature, and aging time different matrices were produced. Both microspheres (spray-dried) and monolithic sticks (at low temperature dried gels, i.e., xerogels) are prepared. Some of these matrices were doped with BSA and preliminary protein release profiles are shown. *In vitro* bioresorption of SiO_2 xerogel matrices was studied *in sink* conditions. Characterisation of the matrices is done by N_2 sorption isotherms.

2. Materials and Methods

2.1. *Preparation of Monolithic Sticks and Microparticles*

SiO_2 xerogel matrices were prepared by the hydrolysis and polycondensation of tetraethoxysilane (TEOS 98 %, Aldrich). Different H_2O/TEOS mole ratios (R), ethanol amounts, pH, and aging times were used for the sols. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used to adjust the pH. The initial pH of the sols were between 2–3 and the pH was raised to 4.4–6.3 before the protein was added into the sols. Bovine serum albumin BSA (bovine albumin extracted from buffalo blood) is used as model protein. It has the molecular weight of 68 000 g/mol. Brown and Shockley (1982) have constructed a model of BSA as having a shape of cigar in size 140 × 40 Å [23].

The sols were divided into 3 groups A, B and C as shown in Table 1. In Group A, the initial pH_1 of the sol is 2.8 and R_1 is 10–30 ($R = H_2O$/TEOS). After the sol hydrolysis the pH_1 was raised to a protein-friendly level (pH_2 = 4.7–5.3). BSA was added into the sol in a water solution, which raises the R_1 to R_2. Matrices, both monolithic sticks and microspheres, were prepared with and without BSA. In Group B, the initial R_1 is only 2, pH_1 is 2 and also ethanol is used in the sol (EtOH/TEOS$_1$ = 1). The goal is to minimise the degree of condensation in order to prepare relatively fast dissolving SiO_2 xerogel matrices. After the hydrolysis, the sols were aged different time periods ranging from 21 to 95 h, at 40°C. 95 hours corresponds to relative gel times, $t_{gel} \approx 0.9$–0.95 ($t_{gel} = 0$ corresponds the preparation of a sol and $t_{gel} = 1.0$ corresponds the gel formation (gel point)). During that time hydrolysis, condensation as well as the particle formation and growth proceed simultaneously and the extent of the processes depends on the particular conditions. After sol aging the sol was diluted (expect the freeze dried sol), and the pH was raised to 4.4–6.9. The sols, which were used to produce microspheres, were kept in an ice bath as the base was added (to avoid too fast gel formation; the sol have to be in the liquid state during the spray drying). In Group C the R_1 was 15 or 30 and the initial pH_1 was 2. The aging time of the sol was between 39–66 h, at 40°C. The pH_2 was raised after aging except in two cases. These matrices (Cm18 and Cm21) are not suitable for proteins. Two matrices (Cm20 and Cm23) with 5 m-% BSA were done. Microspheres were not prepared in Group C.

Monolithic sticks were prepared in a cylindrical teflon moulds (3 × 25 mm). The sol was injected into the moulds where the gelation and drying occurred. Sols and the formed gels were aged and dried at 4°C or at 40°C and 40% relative humidity (see Table 1). No further heat-treatment was used. The drying process of the monolithic sticks at 4 or 40°C is a slow and unforced process done at constant conditions, where the gel structure is formed. In order to shorten the drying time of monolith, one specimen was freeze-dried

Table 1. Sol-gel formulation for monoliths and microspheres.

Sample name	R_1	Sol pH_1	EtOH/TEOS$_1$ (mol)	Hydrolysis time* (min)	Sol aging time (h)	R_2	EtOH/TEOS$_2$ (mol)	Sol pH_2	BSA (m-%)	Drying T (°C)	Spray drying T (°C)
Group A R_1 = 10–30, pH_1 = 2.8											
Monoliths											
Am01	15	2.8	–	170	–	15	–	5.3	–	40	–
Am02	30	2.8	–	110	–	30	–	5.1	–	40	–
Am03	10	2.8	–	170	–	12	–	5.0	5	40	–
Am04	25	2.8	–	110	–	37	–	5.0	5	40	–
Microspheres											
As05	30	2.8	–	110	–	30	–	5.0	–	–	135
As06	30	2.8	–	110	–	34	–	5.0	1.7	–	135
As07	30	2.8	–	110	–	34	–	4.7	5	–	135
Group B R_1 = 2, pH_1 = 2											
Monoliths											
Bm08	2	2	1	15	66	15	1	6	–	4	–
Bm09	2	2	1	15	63	30	1	5.8	–	4	–
Bm10	2	2	1	15	22	15	5.3	6.9	–	4	–
Bm11	2	2	1	15	95	15	1.7	6.6	–	4	–
Bm12	2	2	1	15	65	2	1	6.3	–	freeze drying	–
Microspheres											
Bs13	2	2	1	15	22	15	5.3	6.9	–	–	120
Bs14	2	2	1	15	70	15	5.1	5.7	–	–	120
Bs15	2	2	1	15	90	15	4.3	6.4	–	–	120
Bs16	2	2	1	15	21	17	5.1	4.7	5	–	120
Bs17	2	2	1	15	90	17	5.0	4.4	5	–	120
Group C R_1 = 15 or 30, pH_1 = 2											
Monoliths											
Cm18	15	2	–	20	42	15	–	2.0	–	4	–
Cm19	15	2	–	20	39	15	–	6.3	–	4	–
Cm20	15	2	–	20	39	20	–	6.3	5	4	–
Cm21	30	2	–	20	66	30	–	2.0	–	4	–
Cm22	30	2	–	20	66	30	–	6.2	–	4	–
Cm23	30	2	–	20	66	34	–	6.2	5	4	–

Hydrolysis Time* = time needed to achieve a clear sol, pH_1 = the calculated initial pH of the sol, pH_2 = the adjusted pH for proteins, $R = H_2O/TEOS$, R_1 = the initial R of the sol, R_2 = R after sol dilution and/or BSA adding (BSA was add in water solution), m = monolith; s = microsphere

in vacuum at −10°C. Microspheres were prepared by spraying silica sol with a mini spray dryer (B-191, Büchi Labortechnik AG, Switzerland). The following process parameter were used: pump 16%, aspirator 95%, and flow 600 l/h. The temperature during the microsphere formation is only instantly at elevated level, at 120–135°C (inlet temperature). Solvents are forced to evaporate fast and relatively dry microspheres are obtained.

2.2. *SiO_2 Release*

SiO_2 xerogel matrix resorption is measured in 0.05 M TRIS (Trizma® pre-set Crystals, Sigma) solution

buffered at pH 7.4, $T = 37°C$. The TRIS was sterilized at 121°C before use. The SiO_2 concentration in the TRIS was kept below 30 ppm (to ensure *in sink* conditions; free dissolution of the SiO_2 matrix). The SiO_2 saturation level at pH 7.4 is about 130 ppm. If needed, 75 % of the dissolution medium was changed to the fresh TRIS in order to keep the SiO_2 concentration below 30 ppm. The Si concentration was measured with spectrophotometer (UV-1601, Shimadzu) analysing the molybdenum blue complex absorbance at 820 nm.

2.3. *Protein Release*

BSA release is analysed with the fluorescence method (Photo Technology International) with NanoOrange N-6666 Kit (Molecular Probes). A more diluted TRIS solution (0.005 M) had to be used, because higher TRIS concentrations disturb the protein analysis method. The BSA release test is done at different TRIS volumes at different time points, because otherwise the released protein concentration would have been too low for the protein concentration analysis. This causes the maximum SiO_2 concentration of 79 ppm in the BSA dissolution test (Fig. 3). All the dissolution bottles were kept in the shaking water bath (Comfort Heto Master Shake) at 37°C, speed at 170.

2.4. *N_2 Sorption*

Dried SiO_2 gels have always a great deal of moisture left in the pores, which makes the surface area measurement more difficult. All pores have to be emptied in order to achieve reliable specific surface area results. If the pores are only partly empty the measurement is impossible, because the vacuum inside the measurement chamber cannot be maintained (evaporation of moisture from the specimen). It is possible to achieve indirect results on the pore structure by heat-treating the gels before moisture evacuation, but this will change the pore structure and difference to the dried gels is considered to be too great. Instead of further heat-treatment, we have used 6 hours acetone wash to remove the moisture and ethanol from the pores of the dried SiO_2 gel. Acetone wash is also used by Harreld et al. to remove the water, alcohol, and unreacted monomers from aged gels [24]. After the acetone wash the gels were kept in a desiccator at room temperature. The N_2 sorption isotherms were measured using a Micromeritics ASAP 2010, Brauner, Emmet and Teller (BET) method was used to calculate the specific surface area ($P/P_0 < 0.22$). The evacuation of the specimens was done in the measurement chamber at 30°C. The pressure prior to the measurement was 2–5 μmHg.

2.5. *TG-Measurements*

Thermogravimetric analysis (Netzsch-TA4) was done before and after the acetone wash in order to confirm the moisture evacuation from the pores previous to N_2 sorption measurements. The heat-treatment speed 0.3 K/min was used. The measurements were done from 20 to 600°C. N_2 was used as protective gas.

3. Results and Discussion

3.1. *Pore Structure*

The specific surface area (SSA) of the monolithic sticks could be adjusted on a large scale where as all microparticles have a low SSA. There are no great differences in the average pore diameters (Table 2). Two examples of N_2 adsorption-desorption isotherms are shown in Fig. 1. The N_2 sorption isotherms show that the results for SSA and pore volume are reliable, but in the case of average pore diameter, some of the results are questionable (not shown in Table 2). This depends on the low adsorbed amounts of N_2 in the case of dense specimens resulting in inadequate quality of the sorption isotherm on the area, which is used to calculate the average pore diameter. The TGA results for Cm19 and Cm20 indicate 5–10 % weight loss (water, ethanol and unreacted monomers) after the acetone wash. After the acetone wash it was possible to evacuate the SiO_2 specimens at room temperature, which ensures minimal structural changes during the pre-treatment of the N_2 sorption measurement.

3.2. *SiO_2 Dissolution and Protein Release*

The main results summarising the dissolution of SiO_2 and its connection to SiO_2 matrix structure are shown in Fig. 2. The SiO_2dissolution is shown after 1, 3 and 7 days of immersion in the TRIS solution at 37°C buffered to the body fluid pH = 7.4. It is observed that specific surface area is not the most important factor controlling the dissolution of SiO_2. The microspheres with SSA = 4.3 m^2/g (Bs15) dissolve much faster than

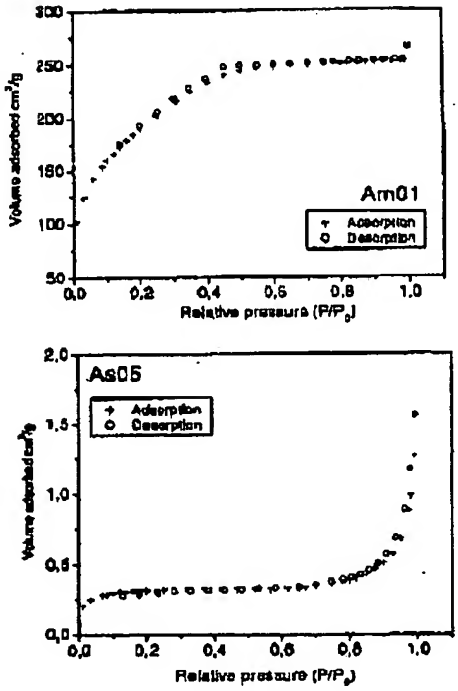

Figure 1. N_2 adsorption-desorption isotherms for the monolith Am01 and microsphere As05. BET surface area is calculated from the isotherm at relative pressure <0.22.

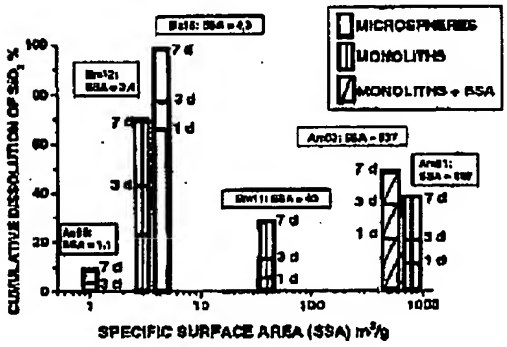

Figure 2. The SiO_2 dissolution as cumulative weight percent after 1, 3, and 7 days versus specific surface area of the specimen.

Table 2. BET surface area, pore volume and average pore diameter calculated from N_2 sorption measurements.

Sample name	BET surface area (m^2/g)	Pore volume (cm^3/g)	Average Pore diameter (nm)
Group A			
Monolith			
Am01	697	0.30	2.2
Am02	672	0.36	2.1
Am03	637	0.34	2.1
Am04	628	0.40	2.5
Microparticles			
As05	1.1	0.001	*
As06	1.3	0.002	*
Group B			
Monolith			
Bm08	451	0.22	1.9
Bm09	518	0.27	2.0
Bm10	382	0.19	2.0
Bm11	42.5	0.02	*
Bm12	3.4	0.001	*
Microparticles			
Bs15	4.3	0.001	*
Group C			
Monolith			
Cm18	4.7	0.001	*
Cm19	426	0.21	2.0
Cm21	6.4	0.003	*
Cm22	479	0.23	2.1

the monolithic stick with SSA = 697 m^2/g (Am01). The main parameters controlling the SiO_2 sol-gel process are well characterised both with respect to the chemical structure [9, 22] and pore structure formation [9]. This information has been utilised in this study to control the SiO_2 structures. The microspheres (Bs15) have been prepared at conditions [22] that result in a low degree of condensation of SiOH (low pH, low R). In the case of the monolithic stick (Am01), the conditions favour the condensation to proceed faster. In both cases the pH is raised from pH 2–3 to the protein-compatible level (pH 5.3 and 6.4) that is known to favour condensation [9], but in the case of microspheres the raised pH is not let to affect the process, because the spray-drying is done right after the pH adjustment. For the monolith the drying occurred slower in the mould favouring the condensation reaction. In addition, the monolithic

stick has been prepared at higher water-to-TEOS ratio, which is known to favour hydrolysis [9]. This shows the importance of the chemical structure of SiO_2 and it is accordance with earlier observations for sol-gel derived silica fibers made at corresponding conditions resulting in a low degree of condensation [25, 26].

The main principle for a protein-friendly sol-gel process for SiO_2 has been introduced earlier [14], but the process was developed only for protein encapsulation, not for (controlled) release. Slower SiO_2 dissolution is easier to achieve, because the main parameter controlling the slow dissolution is the same as the suitable pH for many proteins, pH at ~ 4–7. To achieve fast dissolution at protein-friendly conditions, the sols for microspheres in Group B were first aged near to the gel point (corresponding the relative gel time at 0.90–0.95) at pH = 2 using a low water-to-TEOS ratio (R = 2). This results in a sol where the number of Si-polymers increases, but the rate of condensation stays low. The pH was adjusted to 4.4–7 before protein addition. During the sol aging near to the gel point, the viscosity is raised to a level, where spray-drying is practically impossible and the sol had to be diluted by adding extra water and ethanol into the sol. Both pH and viscosity adjustment affect the formation of chemical structure. Ethanol, which retards the condensation reactions, would be a better solvent to adjust the viscosity than water that favours further hydrolysis and condensation. Unfortunately, the use of ethanol is limited due to danger of explosion during spray-drying in air atmosphere. In addition, the possible denaturation of proteins caused by ethanol has to be taken into the consideration. To retain the fast-dissolving structure achieved during the aging of the sol (in Group B), the sol was spray-dried to microspheres right after the pH adjustment and dilution. In the case of corresponding monoliths, the pH adjustment and dilution caused remarkable changes during the relatively short aging time (compared to the initial sol aging time at low pH and R) between the adjustments and the gel point. SiO_2 dissolution of the monoliths (Bm08–11) was much slower than for corresponding microparticles (Bs13–17) and it is analogical with the group (Cm18–23) that had corresponding conditions from the beginning. Due to the strong effect of pH adjustment and dilution of the sols (group B) on the monolithic sticks, another method was developed to achieve fast dissolution. In analogy with the spray-drying of microspheres, freeze-drying was done right after the pH adjustment (Bm12). The resulting monolith dissolved clearly faster (Table 3).

Table 3. Degradation of SiO_2 matrix in TRIS -solution at T = 37°C and pH = 7.4.

Sample name	SiO_2 dissolution burst level (%)	Burst rate (%/h)	SiO_2 dissolution rate (after burst) (%/h)
Group A			
Monoliths			
Am01	11.6	0.46	0.19
Am02	14.4	0.57	0.23
Am03	21.9	1.00	0.18
Am04	17.8	0.81	0.18
Microspheres			
As05	–	–	0.09
As06	9.38	0.43	0.09
As07	–	–	0.24
Group B			
Monoliths			
Bm08	–	–	0.28
Bm09	–	–	0.34
Bm10	–	–	0.24
Bm11	–	–	0.25
Bm12	25.2	0.93	0.33
Microspheres			
Bs13	100	2.17	–
Bs14	100	2.17	–
Bs15	100	2.17	–
Bs16	100	2.27	–
Bs17	100	2.27	–
Group C			
Monoliths			
Cm18	–	–	0.13
Cm19	–	–	0.24
Cm20	–	–	0.27
Cm21	–	–	0.19
Cm22	–	–	0.24
Cm23	–	–	0.22

The effect of both chemical structure and the specific surface area on the monoliths is seen in Fig. 2. Bm11 (SSA = 43 m^2/g) and Bm12 (SSA = 3.4 m^2/g) are both monolithic sticks with relatively low surface area, but difference in SiO_2 dissolution is clear and, in analogy with the microspheres, the denser matrix, Bm12, dissolves faster. The reason is the same as for the microspheres. Bm12 has been prepared at conditions that

result in low degree of condensation of SiOH and the freeze-drying is done right after the pH and R adjustments. The specimen (Bm11) with SSA 43 m^2/g is initially aged at corresponding conditions, but after that the R is adjusted to 15 and pH to 6.6, which favour condensation of SiOH during the slower, spontaneous aging and drying. Although the raised pH affected also the formed pore structure (one order of magnitude larger SSA than for SiO_2 with SSA = 3.4 m^2/g), the dissolution of SiO_2 was still clearly slower. The specimen with SSA 697 m^2/g (Group A, Am01) is prepared from the beginning at conditions that correspond those of Bm11 after pH and R adjustment. The hydrolysis is done at pH2.8 and R = 15 (Table 1) and pH was adjusted to 5.3 right after the hydrolysis. Thus, as the monolithic sticks SSA = 43 m^2/g and SSA = 697 m^2/g (both affected by conditions that favour condensation) are compared to each other, the difference in the dissolution rate is due to the differences in the specific surface area and pore volume. However, the effect is weaker than that observed between Bm11 and Bm12.

Although the freeze-drying showed its potential in the preparation of fast-dissolving monoliths at protein-compatible conditions, the quality of the freeze-dried monolithic stick needs to be studied further due to a brittle structure in some specimens. Another possibility to prepare fast-dissolving monoliths (that are not brittle) is the process done at pH ~ 2 and at R ~ 2, as shown earlier for corresponding SiO_2 fibers [25,26] but the low pH is a problem without additives that protect the biological activity of proteins.

If one compares two microspheres groups to each other (Group A and B), the difference in SiO_2 dissolution (Table 3) is also clear, but there is not so big difference in the specific surface area or pore volume (Table 2). The dissolution results point out again the importance of the chemical structure.

The influence of added protein, BSA, on the SiO_2 dissolution is also shown in Fig. 2. The monolithic stick containing BSA with SSA = 637 m^2/g (Am03) and the corresponding pure SiO_2 stick with SSA = 697 m^2/g (Am01) were both prepared using parameters that result in large surface areas. The specific surface area is a bit lower for the specimen containing BSA, but the order of magnitude is still the same. The BSA-containing specimen dissolves faster and the difference, caused mainly by the difference in the initial dissolution (burst, Table 3), is assigned to the extra space in the pore structure caused by the presence of BSA. The similarity of SSA results suggests also that BSA does not significantly disturb the reactions or aggregation of Si species.

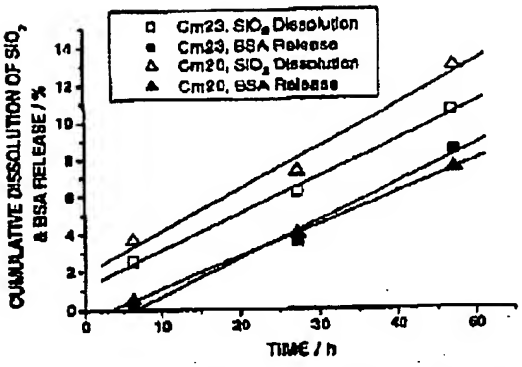

Figure 3. Dissolution of SiO_2 matrix and release of BSA-protein from the matrix for specimens Cm20 and Cm23.

SiO_2 dissolution and BSA release for two monolithic sticks prepared similarly, but having a difference in water-to-TEOS ratio (R = 15 and R = 30) (Cm20 and Cm23), are shown in Fig. 3. The differences between SiO_2 dissolution and BSA release are not great. The SiO_2 dissolution is in both cases a bit faster than BSA release, but the slope of the linear fits (correlation in all cases > 0.99) agree almost perfectly. Although the SiO_2 dissolution has a slight burst, the BSA release is slow in the beginning. This is suggested to occur due to SiO_2 dissolution that is needed to release the first encapsulated protein molecules nearest to the surface. The BSA release was modelled with empirical equation [27, 28] $M_t/M\infty = kt^n$, were $M_t/M\infty$ is the fractional release amount of drug, k is constant, t is time and n is diffusional exponent. For classical Fickian diffusion n is 0.5 and for erosion controlled system (zero order release) n is 1. In our case $n > 1$, which originates from the lag time in the beginning. The linear release after initial slow phase, however, indicates a release system that is not diffusion-controlled. The results on the pore structure for the corresponding monoliths in Group C show that the average pore diameter is smaller than the size of BSA, which supports the suggestion on the matrix-erosion controlled release.

The overall dissolution rate of monoliths is faster in Group A compared to the Group B and C and it depends mostly on the initial burst up to ~25 h (Table 3). The main difference compared with the other groups

is the high surface area and pore volume (Table 2) that seems to favour the burst. The effect of burst is even more significant in the BSA-doped specimens and it depends also on the loading of BSA (microspheres in Group A). The dissolution rate of SiO_2 was slowest for pure SiO_2 microspheres and increased as the loading is increased from 1.7 to 5 m-% BSA. In Group C there are two matrices where the pH was not raised after the hydrolysis. These matrices (pH = 2) had a bit slower SiO_2 dissolution rate than the other specimens in Group C. This is partly due to denser pore structure (relatively fast hydrolysis, but slower condensation). There was not a great difference in SiO_2 release in the protein-doped and undoped monoliths prepared at $pH_2 = 6$, $R_1 = 15$ or $R_1 = 30$. This leads to a logical conclusion (in group C) that the doped BSA disturbs less SiO_2 specimens with a larger surface area than SiO_2 specimens with smaller surface area.

As the surface area-to-volume (SA/V) of the studied monolithic sticks and microspheres are compared to each other (either by using external surface areas and volumes or the measured values for pore structure in Table 2), it is clear that the obtained SA/V values (values within the same order of magnitude for all specimens by using the measured surface area/g and pore volume/g and by using SA for 1 g = SSA compared to external volume, the SA/V is much larger for slowly dissolving specimens) do not explain the great differences in the dissolution rates.

4. Conclusion

Bioresorption (dissolution at the body fluid pH) of SiO_2 matrices, both dried monolithic sticks and spray-dried microspheres, can be adjusted on a large scale within a narrow protein-friendly sol pH-window. Simple adjustments of sol component concentrations and sol aging resulted both in fast (days) and slow (weeks & months) dissolution. The fastest dissolving matrix was achieved for one of the densest matrices (SSA = 4.3 m^2/g and pore volume = 0.001 cm^3/g), which dissolved clearly faster than the specimen with 162 times higher specific surface are (SSA = 697 m^2/g and pore volume = 0.39 cm^3/g) pointing out that the importance of chemical structure of SiO_2 in comparison with the specific surface area and pore volume.

It was shown that the fast dissolution of SiO_2 matrices is retained if the properly aged sol at low pH and R-value is quickly dried (spray- or freeze-dried) after pH adjustment to the protein-friendly level. Fast drying prevents extensive condensation of SiOH groups. The slow resorption is achieved both for monoliths and microspheres by simple adjustments of sol component concentrations.

BSA is released from the SiO_2 matrix and the release profiles are linear. Diffusion is not observed due to large size of proteins. For matrices prepared at low pH and R-value, the protein release and matrix resorption nearly overlap suggesting nearly surface eroding matrice. The results show additionally that the added BSA does not significantly disturb the SiO_2 matrix formation and it affects less the resorption results of SiO_2 as the matrices have a larger surface area.

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